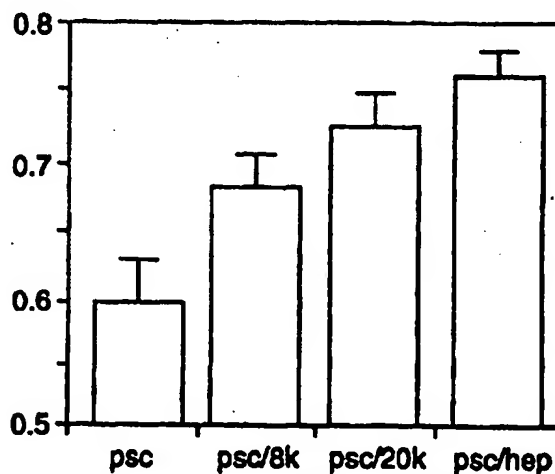




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61L 15/28, 15/64		A1	(11) International Publication Number: WO 98/00180
			(43) International Publication Date: 8 January 1998 (08.01.98)
(21) International Application Number: PCT/GB97/01725		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 27 June 1997 (27.06.97)		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(30) Priority Data: 9613682.5 28 June 1996 (28.06.96) GB			
(71) Applicant (for all designated States except US): JOHNSON & JOHNSON MEDICAL, INC. [US/US]; 2500 Arbrook Boulevard, Arlington, TX 76004-3030 (US).			
(72) Inventors; and (73) Inventors/Applicants (for US only): WATT, Paul, William [GB/GB]; 9 Beck Side, Skipton BD23 3ET (GB). HARVEY, Wilson [GB/GB]; 53 Westwood, Carlton BD23 3DW (GB). LORIMER, Elaine [GB/GB]; 68 Grangeneuk Gardens, Balloch, Cumbernauld G69 9BP (GB). WISEMAN, David [US/US]; 135 Amherst Street, Highland Park, NJ 08904 (US).			
(74) Agent: FISHER, Adrian, John; Carpmiels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).			

(54) Title: USE OF OXIDIZED CELLULOSE AND COMPLEXES THEREOF FOR CHRONIC WOUND HEALING



BEST AVAILABLE COPY

(57) Abstract

The invention provides the use of oxidized cellulose, preferably oxidized regenerated cellulose (ORC), and complexes thereof with structural proteins such as collagen, for the preparation of wound dressings for the treatment of chronic wounds. Preferably, the dressing is a knitted, woven or nonwoven fabric of ORC suitable for application directly to the surface of a wound as a wound dressing, or a semi-solid ointment for topical application containing dispersed ORC fibers or powder, or a collagen/ORC sponge. Preferably, the chronic wound is a venous ulcer, a decubitus ulcer or a diabetic ulcer.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

USE OF OXIDIZED CELLULOSE AND COMPLEXES THEREOF
FOR CHRONIC WOUND HEALING

The present invention relates to the use of oxidized
5 celluloses and complexes thereof with structural proteins
such as collagen for chronic wound healing.

Oxidized cellulose is produced by the oxidation of
cellulose, for example with dinitrogen tetroxide. This
10 process converts primary alcohol groups on the saccharide
residues to carboxylic acid group, forming uronic acid
residues within the cellulose chain. The oxidation does not
proceed with complete selectivity, and as a result hydroxyl
groups on carbons 2 and 3 are occasionally converted to the
15 keto form. These ketone units introduce an alkali labile
link, which at pH 7 or higher initiates the decomposition of
the polymer via formation of a lactone and sugar ring
cleavage. As a result, oxidized cellulose is biodegradable
and bioabsorbable under physiological conditions.

20

The preferred oxidized cellulose for practical
applications is oxidized regenerated cellulose (ORC)
prepared by oxidation of a regenerated cellulose, such as
rayon. It has been known for some time that ORC has
25 haemostatic properties. ORC has been available as a
haemostatic product called SURGICEL (Registered Trade Mark
of Johnson & Johnson Medical, Inc.) since 1950. This
product is produced by the oxidation of a knitted rayon
material.

30

A modification of porosity, density and knit pattern
led to the launch of a second ORC fabric product, INTERCEED
(Registered Trade Mark - Johnson & Johnson Medical, Inc.)
which was shown to reduce the extend of post-surgical
35 adhesions in abdominal surgery.

US-A-2517772 (Doub et al.) describes improved haemostatic materials obtained by impregnating ORC fabric with thrombin.

5 EP-A-0437095 describes a neutralised ORC material prepared by contacting an as-synthesised acidic ORC material with a solution of a basic salt of a weak organic acid, such as sodium acetate. The resulting neutralised product is indicated for haemostasis and adhesion prevention.

10

Collagen, which is a structural protein of animal origin, is known in various forms for use as a wound dressing material.

15 GB-A-1515963 describes cross-linked collagen-mucopolysaccharide composite materials for use in medical and surgical applications, blood vessel grafts and all forms of surgical prostheses. The composite material contains at least 0.5% by weight of a mucopolysaccharide irreversibly
20 bound to collagen. The mucopolysaccharide is an animal polysaccharide containing hexosamine residues, such as hyaluronic acid, chondroitin sulphate or heparin sulphate. The composite materials are said to exhibit greater resistance to resorption and better blood compatibility than
25 simple collagen materials.

US-A-4614794 describes complexes formed between collagen and polyanionic plant polysaccharides, such as sodium alginate. The complexes are preferably formed by
30 combining the protein and the polysaccharide at a pH which is no higher than the isoelectric point of the protein. The resulting complexes are said to be suitable for a wide variety of medical and surgical applications, including wound dressings. There is no disclosure of the use of
35 oxidized cellulose instead of the polyanionic plant polysaccharide. The specification also teaches that

proteins other than collagen, such as fibrin or elastin may be used in the formation of useful protein/polysaccharide complexes.

- 5 GB-A-2280850 describes medicated implants for the treatment of periodontal disease comprising a medicated collagen film reinforced with a layer of bioresorbable polymer, which may be ORC. The collagen matrix may contain dispersed ORC fibers or fragments.

10

The above-described collagen or collagen/polysaccharide wound dressing materials provide important advantages. The materials are of natural, biological origin (albeit sometimes chemically modified), and consequently tend to
15 have low antigenicity. The materials are generally bio-absorbable, which reduces the trauma associated with removal of conventional wound dressing materials from the surface of the wound. Furthermore, some of these materials can have positive therapeutic effects on wound healing. For example,
20 some animal mucopolysaccharides such as hyaluronic acid are thought to exert a chemotactic effect on wound healing cells such as fibroblasts, and thereby promote the growth and development of such cells. Nevertheless, there remains a need for improved wound dressing materials of this general
25 type exhibiting still better control of physical properties and biological absorption rates, still better therapeutic effects on wound healing, and reduced cost.

It is an object of the present invention to provide
30 improved wound dressing materials for mammalian, and especially human, chronic wounds, such as venous ulcers, decubitis ulcers and diabetic ulcers. Such chronic wounds generally exhibit little or no bleeding or adhesion to other body tissues, and accordingly oxidized cellulose would not
35 previously have been indicated for the treatment of such wounds.

It has now been found that oxidized cellulose is an effective wound dressing material for the treatment of chronic wounds.

5

Accordingly, the present invention provides the use of oxidized cellulose for the preparation of a wound dressing material for the treatment of a chronic wound.

10 Preferably, the oxidized cellulose is oxidized regenerated cellulose (ORC). Preferably, the oxidized cellulose is in the form of a woven, non-woven or knitted fibrous body that is insoluble in wound fluids. For example, the oxidized cellulose may be in the form of one of
15 the SURGICEL (RTM) or INTERCEED (RTM) fabrics known in the art. In other preferred embodiments, the oxidized cellulose is in the form of fibers, such as milled fibers, or powder, preferably dispersed in a suitable topical medicament vehicle.

20

Preferably, the oxidised cellulose has an average molecular weight greater than 50,000. Such oxidised cellulose is substantially insoluble in wound fluids, but will undergo very gradual breakdown into bioresorbable
25 fragments at physiological pH.

Preferably, the oxidized cellulose is not neutralized. However, the present invention encompasses the use of partially or completely neutralised materials as described
30 in EP-A-0437095 for the preparation of medicaments for the treatment of chronic wounds as hereinbefore defined.

Preferably, the oxidized cellulose is complexed with a structural protein in the said wound dressing material.

35

Preferably, the protein (where present) and oxidized

cellulose together make up at least 75% by weight of the wound dressing material, more preferably at least 90% by weight of the material. Other components of the material may include 0-25% by weight of one or more other

5 biocompatible polysaccharides, for example alginates such as sodium alginate or calcium alginate, starch derivatives such as sodium starch glycolate, cellulose derivatives such as methyl cellulose or carboxymethyl cellulose, or

10 glycosaminoglycans such as hyaluronic acid or its salts, chondroitin sulfate or heparan sulfate. The material may also comprise up to 20% by weight, preferably up to 10% by weight of water. The material may also contain 0-40% by weight, preferably 0-25% by weight of a plasticiser, preferably a polyhydric alcohol such as glycerol. The

15 material may also comprise 0-10% by weight, preferably 0-5% by weight of one or more therapeutic wound healing agents, such as non-steroidal anti-inflammatory drugs (eg. acetaminophen), steroids, antibiotics (eg. penicillins or streptomycins), antiseptics (eg. silver sulfadiazine or

20 chlorhexidine), or growth factors (eg. fibroblast growth factor or platelet derived growth factor).

Preferably, the weight ratio of protein to oxidized cellulose is from 1:99 to 99.99:1. More preferably, the

25 weight ratio is in the range 1:10 to 99.9:1, still more preferably it is in the range 2:1 to 95:1.

The material according to the present invention may be in any convenient form, such as a powder, microspheres,

30 flakes, a mat or a film. However, preferably, the material according to the present invention is in the form either of a semisolid or gel ointment for topical application, or of a freeze-dried or solvent-dried sponge.

35 Accordingly, the present invention also provides a sterile packaged semisolid or gel ointment for topical

application to a chronic wound, wherein said ointment comprises from 0.05% to 50% w/v of oxidized cellulose.

Preferably, the ointment comprises from 0.1% to 20%
5 w/v, more preferably 1% to 10% w/v, of the oxidized cellulose in a pharmaceutically acceptable carrier. Suitable carriers include: Hydrogels containing cellulose derivatives, including hydroxyethyl cellulose, hydroxymethyl cellulose, carboxymethyl cellulose, hydroxypropylmethyl
10 cellulose and mixtures thereof; and hydrogels containing polyacrylic acid (Carbopols). Suitable carriers also include creams/ointments used for topical pharmaceutical preparations, e.g. creams based on cetomacrogol emulsifying ointment. The above carriers may include alginate (as a
15 thickener or stimulant), preservatives such as benzyl alcohol, buffers to control pH such as disodium hydrogen phosphate/sodium dihydrogen phosphate, agents to adjust osmolarity such as sodium chloride, and stabilisers such as EDTA.

20

Preferably, the oxidized cellulose in the ointment is complexed to a protein, as described herein.

The present invention further provides a freeze-dried
25 or solvent-dried bioabsorbable sponge for application to a chronic wound, the sponge comprising from 0.1% to 50% w/w of oxidized cellulose and from 50% to 99.9% w/w of one or more structural proteins. Preferably, the average pore size of the sponge is in the region of 10-500 μm , more preferably
30 about 100-300 μm .

Suitable proteins for complexation to oxidized cellulose in this invention include the structural proteins such as fibronectin, fibrin, laminin, elastin and collagen.
35 Preferably the protein comprises collagen, and more preferably it consists essentially of collagen. The collagen

may be collagen obtained from any natural source, including microbiological sources, but is preferably collagen obtained from bovine corium that has been rendered largely free of non-collagenous components, for example fat, non-collagenous proteins, polysaccharides and other carbohydrates as described in US Patents Nos. 4614794 and 4320201, the entire contents of which are hereby incorporated by reference. The collagen may be Type I, II or III collagen, or may also be chemically modified collagen, for example an atelocollagen obtained by removing the immunogenic telopeptides from natural collagen. The collagen may also comprise solubilised collagen or soluble collagen fragments having molecular weights in the range 5,000-100,000, preferably 5,000-50,000 obtained by pepsin treatment of natural collagen in known fashion.

Preferably, the oxidized cellulose comprises oxidized regenerated cellulose (ORC). The oxidized regenerated cellulose (ORC) can be obtained by the process described in US Patent No. 3122479, the entire content of which is incorporated herein by reference. This material offers numerous advantages including the features that it is biocompatible, biodegradable, non-immunogenic and readily commercially available. ORC is available with varying degrees of oxidation and hence rates of degradation. The ORC may be used in the form of insoluble fibers, including woven, non-woven and knitted fabrics. In other preferred embodiments, the ORC is in the form of water-soluble low molecular weight fragments obtained by alkali hydrolysis of ORC.

The ready availability of both collagen and ORC having a range of controllable properties means that the properties of the materials used in the present invention can be controlled to an exceptional degree. In particular, the rate of biological absorption, porosity and density of the

materials can be controlled.

It has also been found, surprisingly, that the protein/oxidized cellulose complexes used in the preferred aspect of the present invention have an excellent ability to bind to growth factors, in particular, platelet derived growth factor. Accordingly, the present invention also provides the use of oxidized cellulose or complexes thereof with a structural protein to bind one or more cell growth factors. Preferably, the cell growth factor is platelet derived cell growth factor (PDGF).

The present invention further provides a method of separating cell growth factors from a biological sample or organism, the method comprising:

- (i) contacting the biological sample or organism with a material comprising oxidized cellulose or a complex thereof with a structural protein as hereinbefore defined, the contacting being carried out *in vivo* or *in vitro*, to bind the growth factors to the material; and
- (ii) recovering the bound growth factors from the material.

The present invention further provides a method of preparing an active wound dressing material comprising the step of:

- (i) contacting oxidized cellulose or a complex thereof with a structural protein as hereinbefore defined with a biological medium containing cell growth factors to bind the cell growth factors to the material;
- (ii) washing and drying the material having the cell growth factors bound thereto to form said active wound dressing material. Preferably, the cell growth factors comprise platelet derived growth factor.

In another aspect, the present invention provides a method of treatment of a chronic wound in a mammal, such as

a decubitis ulcer, a venous ulcer or a diabetic ulcer. The method comprises applying a dressing comprising oxidized cellulose or a complex thereof with a structural protein as hereinbefore defined to the wound.

5

Preferably, the oxidized cellulose is applied to the chronic wound for a period of at least 1 hour, more preferably at least 6 hours, and most preferably at least 12 hours. The oxidized cellulose treatment may be extended for
10 several days or weeks, with oxidized cellulose dressing changes as appropriate, if necessary for chronic wounds. This contrasts with haemostatic applications of ORC, which typically last only a few seconds or minutes.

15 Without wishing to be bound by any theory, it is thought that the oxidized cellulose and complexes thereof promote chronic wound healing in at least some of the following ways. Firstly, the oxidized cellulose binds to growth factors such as PDGF, EGF and FGF to retain these
20 growth factors at the wound site. Otherwise, such growth factors tend to be carried away from the wound site along with the wound exudate. The gradual breakdown of oxidized cellulose at physiological pH results in gradual release of the growth factors back into the wound. A second reason is
25 that oxidized cellulose is fully bioresorbable and physiologically acceptable. A third reason may be that the oligosaccharide fragments produced by the breakdown of oxidized cellulose in vivo themselves promote chronic wound healing.

30

Preferably, the chronic wound is selected from the group consisting of venous ulcers, decubitis ulcers and diabetic ulcers. Preferably, the chronic wound is substantially or completely non-bleeding. The term "chronic
35 wound" does not encompass a periodontal disorder or disease.

The protein/oxidized cellulose complexes used in the present invention can be made by a process comprising the steps of: providing an aqueous dispersion of a protein; immersing or dispersing oxidized cellulose in the aqueous dispersion; following by removing water from the aqueous dispersion to leave a material comprising protein complexed with oxidized regenerated cellulose.

The optional, additional components in the materials according to the present invention are preferably included in the aqueous dispersion prior to removal of water from the aqueous dispersion.

Preferably, the pH of the dispersion is adjusted to pH 3-4.5. This pH range is less than the isoelectric pH of collagen.

The water can be removed from the aqueous dispersion by evaporation, for example by evaporation from the dispersion in a tray to leave a film of material. However, preferably, the water is removed by freeze-drying (lyophilizing) or solvent-drying to produce the material in the form of a sponge. Preferably, the aqueous dispersion contains 5-30mg/ml of collagen. Preferably, a collagen-based sponge is formed by lyophilisation substantially as described in US-A-2157224.

Preferably, the process further comprises treating the protein and polysaccharide in the dispersion, or in the dried material, with a cross-linking agent such as carbodiimide, hexamethylene diisocyanate (HMDI) or glutaraldehyde.

Alternatively, cross-linking may be carried out dehydrothermally. The method of cross-linking can markedly affect the final product. For example, HMDI cross-links the primary amino groups on the protein within the complex,

whereas carbodiimide cross-links carbohydrate on the ORC to primary amino groups on the protein.

5 The oxidized cellulose may be added to the aqueous dispersion of protein in the form of a suspension or solution of the oxidized cellulose, preferably at a comparable pH to the collagen suspension, following by mixing by stirring or homogenisation. Alternatively, dry
10 fibers or fabric of oxidized cellulose may be immersed in the aqueous dispersion of collagen.

Specific embodiments of the present invention will now be described further, by way of example, with reference to
15 the accompanying drawings, in which:

Figure 1 shows a graph of fibroblast cell growth (in arbitrary units) on serum treated films consisting of (a) pepsin solubilised collagen, (b) pepsin solubilised collagen
20 complexed with ORC fragments having average molecular weight 8,000, (c) pepsin solubilised collagen complexed with ORC fragments having average molecular weight about 20,000, and (d) pepsin solubilised collagen complexed with heparan sulphate (for comparison);

25 Figure 2 shows a graph of percentage binding of platelet derived growth factor (PDGF) to the following materials: (a) plastics, (b) collagen sponge, (c) collagen/solubilised ORC sponge, (d) INTERCEED (RTM) ORC, and the ease of removal of this growth factor from the
30 materials; and

Figure 3 shows a graph of relative amounts of MMP binding measured for a collagen sponge and SURGICEL (RTM) ORC fabric (comparative measurements) and for sponges of collagen complexed with 10 wt.%, 20wt.% and 30 wt.% of
35 fibrous ORC.

Example 1: Preparation of a collagen/fibrous ORC sponge

Lyophilised collagen, prepared as described in US Patent No. 4614794 of 4320201, is re-suspended in cold 0.05M acetic acid at a concentration of 10mg/ml. Milled ORC powder (milled Surgicel® cloth) is added to the suspension in a ratio of 1:3 ORC:collagen and homogenised using a Waring Blendor on low speed for 3 x 30s. The complex suspension is degassed in a vacuum oven for 10 min. and then poured to a depth of 3mm and blast frozen. The frozen suspension is then either freeze-dried and dehydrothermally cross-lined using a programmable Edwards freeze-drier with a temperature ramping facility, or dried using a solvent drying process as described in US-A-2157524.

15

Example 2: Preparation of a collagen/ORC oligosaccharide sponge

Soluble collagen is prepared by the published method of E.J. Miller and R.K. Rhodes "Preparation and Characterisation of the Different Types of Collagen", Methods Enzymol Vol. 82, pages 33-64 (1982). Soluble oligosaccharides of ORC are prepared as described in a co-pending Patent Application filed on the same date as this application and commonly assigned herewith. Briefly the ORC oligosaccharides are prepared by treating commercially available ORC with 6M sodium hydroxide solution at 37°C for 45 minutes, followed by neutralization and dialysis to remove fragments and impurities having molecular weight below 1000. The resulting soluble oligosaccharides of ORC are slowly added while stirring to soluble collagen (.075mg/ml) (both in cold 0.05M acetic acid) until no further precipitation of the complex occurs. The complex precipitate is isolated by centrifugation, washed in phosphate buffer at pH 7.2, and re-suspended, by homogenisation, at 30% w/v in the same buffer. The suspension is poured to a depth of 3mm, blast frozen at

-30°C and freeze-dried.

Example 3: Preparation of a collagen/ORC film

A collagen/ORC film for application to a wound is made
5 by the methods described in either of the first two
examples, except that the suspensions are not precipitated
but air-dried rather than frozen and freeze-dried. To
prepare flexible films a small amount of glycerol may be
added to the suspensions.

10

Example 4: Preparation of collagen/ORC sponge using a
pre-gelling process of the ORC

ORC fabric (SURGICEL) is suspended (4% w/v) in dilute
alkali (NaHCO₃) at pH 8.0 for a time that is sufficient to
15 convert the fabric to a gelatinous mass. Collagen slurry is
added at the same pH to give a final solids content of both
Surgicel® and collagen of 1% w/v. The slurry is stirred and
the pH adjusted to pH 3.0-4.0 using acetic acid. The final
slurry is moulded, frozen and freeze-dried under vacuum.

20

Example 5

A wound dressing suitable for application to a venous
ulcer, decubitis ulcer or diabetic ulcer is prepared by
cutting 5cm x 5cm² of INTERCEED (RTM) fabric obtained from
25 Johnson & Johnson Medical, Inc.

Example 6

A wound treatment gel for topical application to a
wound is prepared as follows.

30

SURGICEL (RTM) fabric is milled through a 1mm screen
and the resulting fibers are dispersed at 3.0% w/w
concentration in a 3.0% w/w carboxymethyl cellulose (CMC)
aqueous gel. The gel is packaged in a metallized polymer
35 pouch and sterilized by γ -irradiation.

Example 7

A neutralized ORC wound dressing suitable for application to a venous ulcer, decubitis ulcer or diabetic ulcer was prepared as described in EP-A-0437095. Briefly, a quantity of 71.9 grams of oxidized regenerated cellulose (ORC) fabric with a carboxylic acid content of 15.2% available as INTERCEED (RTM) from Johnson & Johnson Medical, Inc., was wrapped around a perforated core and placed in a reactor equipped with a circulating pump. The reactor was filled with a mixture of 3 liters of water 2 liters of methyl alcohol. A Brookfield EX-100 constant temperature bath with built-in pump for external circulation of solutions was turned on to pump the solvent through the centre of the perforated core and through the piles of cloth wrapped around the core. The solvent flowed through the exit line and recirculated again through the pump and back to the core.

A stoichiometric amount of sodium acetate trihydrate equal to the number of moles of carboxylic acid on the cloth was added to the solution, i.e. $71.9 \times 15.2\% = 10.92$ grams of carboxylic acid; equivalent to 33.05 grams of sodium acetate trihydrate.

The 33.05 grams of sodium acetate trihydrate were added to the aqueous alcohol solution and circulated by pump around and through the fabric for 30 minutes. After 30 minutes the pH of the circulating solution reached a constant value (pH 4.6). The fabric was then removed from the reactor and placed in 600 ml of methanol for 10 minutes. the methanol was removed and replaced with another 600 ml of fresh methanol. After the second wash, the cloth was hung up and air dried. A 5cm x 5cm piece of the cloth was cut for use as a wound dressing in accordance with the present invention.

The advantageous properties of the materials according to the present invention prepared as above were determined as follows:

5 Procedure 1: Promotion of fibroblast cell growth

A collagen/alkali-soluble ORC complex film was prepared in a Petri dish as described in Examples 2/3, and serum poured over the film and incubated at 37°C overnight. The serum was removed and the effects on fibroblast cell growth measured (Fig. 1). Cell growth was observed for a pepsin solubilised collagen (PSC) film (control), PSC plus ORC oligosaccharides having average molecular weights of about 8,000 and about 20,000 prepared as described above, and PSC plus heparin was included as a positive control. The results show that the collagen/ORC fragments film appears to bind factors from the serum which stimulate cell growth.

Procedure 2: Binding of platelet derived growth factor

PDGF binding studies were carried out as follows:

20 Small sections of test material (approximately 1cm² squares of INTERCEED (RTM) ORC fabric, and approximately 1cm x 0.5cm x 0.4cm sections of collagen sponge) were weighed and soaked in 100mM sodium phosphate dibasic buffer containing 150mM sodium chloride (total volume 1ml) for at least one hour at room temperature. Samples were then incubated with 2% bovine serum albumin (BSA) in phosphate buffered saline (PBS) for 2 hours at room temperature. 22ng of PDGF was then added to each sample in 250μl of PBS containing 2% BSA, and samples were then incubated for a further hour at 37°C. Each sample was then washed three times with 250μl PBS, followed by increasing concentrations of sodium chloride. Finally, each sample was washed with 4.0M urea. PDGF ELISA analyses of the original PDGF preparation and the various washings were carried out. The data shown in Fig. 2 indicate that the growth factor can be totally recovered from the composite of collagen and ORC while the individual components appear not

to release all the growth factor. The binding characteristics are also uniquely different for the collagen/ORC complex compared with the individual components. These observations indicate that the complex
5 has unique binding of PDGF which may be utilised appropriately for both exogenous binding and endogenous binding and release of growth factor.

Procedure 3: Matrix Metalloproteinase Binding

10 The effect of complexation between collagen and ORC on matrix metalloproteinase (MMP) binding was assessed as follows.

Collagen/fibrous ORC sponges containing 0%, 20% and 30%
15 by weight of fibrous ORC were prepared by the procedure described in Example 1. An ORC-free collagen sponge was prepared for comparison purposes. A sample of SURGICEL (RTM) ORC fabric was also prepared for comparison.

20 Briefly 50mg of each material was placed in a 15ml plastic beaker containing 2.5ml of an acute wound fluid diluted to 1:50 in a proteolysis buffer (50mM tris/HCl pH7.8, 50mM CaCl₂, 0.5M NaCl) and incubated at 37°C on a shaking water bath for 3 hours. Acute wound fluid contains
25 various proteinases, including matrix metalloproteinases and many of these enzymes will preferentially bind to various dressing materials. The excess fluid absorbed by each material was mechanically expressed using a metal spatula and discarded. The remaining dressings were placed into
30 pre-packed 2ml syringes (each syringe contained 0.5ml volume of 2.5mm glass beads). 4ml of proteolysis buffer was forced through the syringe in 1ml aliquots which were discarded. At this washing stage all of the unbound proteinases and proteinases which were only weakly bound to the dressing
35 material had been removed from the dressing leaving the more tightly bound forms. The buffer rinsed dressings were then

removed to another 15ml plastic beaker. 1ml of non-denaturing sample buffer (6.3ml 0.05M tris/HCl.c pH6.8, 2.5ml glycerol, 0.5g SDS, 16.2ml water and bromophenol blue) was added to each sample which were placed on an orbital shaker at setting six for 2 hours. The sample buffer detaches the tightly bound proteinases from the materials which are then present in the sample buffer itself. After this time 20 microliters of sample buffer was taken from each container and subjected to gelatin substrate SDS-polyacrylamide gel electrophoresis (zymography), as described by Heussen C. and Dowdle E.B., Anal. Biochem. 102:196-202 (1980).

The areas of the individual zones of clearance on the gels, which are due to proteinase activity, were accurately measured by the Optilab® system. This was achieved by repeating each binding experiment (n=3) and analyzing the results statistically by the Students T test, where $P \leq 0.05$. Analysis was against controls of pure collagen.

The results, shown in Figure 3, demonstrate a surprising synergistic improvement in MMP binding for the complexes of collagen with ORC. Data are presented for the proenzyme forms (PR02 and PR09) of matrix metalloproteinase 2 (Gelatinase A) and matrix metalloproteinase 9 (Gelatinase B). Without wishing to be bound by an theory, it is thought that the improvement may be related to neutralization of opposing electrostatic charges on the collagen and the ORC by complexation.

Procedure 4

The binding of purified growth factors to pure ORC and neutralised ORC cloth was investigated as follows. Samples of 200 micrograms of PDGF, EGF and FGF were added to 1cm x 1cm squares of INTERCEED (RTM) and neutralised Interceed (from Example 2) and the amount of unbound growth factor was

measured by HPLC. The study investigated both the effect of ionic strength and the effect of carboxylation upon the growth factor binding. The ORC materials were incubated with growth factor for 1 hour at 37°C, then washed with 2 x 5 1 ml of 0.05 or 0.03M NaCl for 5 minutes. The wash solutions were collected, pooled and assayed for growth factor by HPLC. The results are shown in Table 1.

TABLE 1

10	Interceed™	pH	% COOH	% EGF Bound		% FGF Bound		% PDGF Bound	
				Low Salt	High Salt	Low Salt	High Salt	Low Salt	High Salt
15	Interceed™	2.0	15%	97	87	100	100	97	95
	nTC7	5.4	15%	0	0	0	98	84	99
20	Interceed™	2.0	6%	97	33	91	89	98	64
	nTC7	5.4	6%	0	0	100	84	100	84

The results show that between 85 and 100% PDGF, FGF and EGF bind to INTERCEED. However, only FGF and EGF bind to neutralised INTERCEED. It can be postulated that EGF does 25 not bind because of the difference in isoelectric points of the growth factors (PDGF = 9.6, FGF = 10 and EGF = 4.8).

The above examples are intended for the purpose of illustration only. Many other embodiments falling within 30 the scope of the accompanying claims will be apparent to the skilled reader.

CLAIMS

1. Use of oxidized cellulose for the preparation of a medicament for the treatment of a chronic wound.
- 5 2. Use according to Claim 1, wherein the oxidized cellulose is in the form of a woven, nonwoven or knitted fibrous body, that is insoluble in wound fluids.
- 10 3. Use according to any preceding claim, wherein said oxidized cellulose is in the form of dispersed fibers or powders.
- 15 4. Use according to claim 3, wherein said fibers or powder are dispersed in a semi-solid vehicle for topical application.
- 20 5. Use according to any preceding claim, wherein the oxidized cellulose has average molecular weight greater than 50,000.
- 25 6. Use according to any preceding claim, wherein the oxidized cellulose comprises oxidized regenerated cellulose (ORC).
7. Use according to any preceding claim, wherein the oxidized cellulose is complexed with a structural protein.
8. Use according to any preceding claim 4, wherein the oxidized cellulose and structural protein (where present) together make up at least 75% by weight of the material.
- 30 9. Use according to claim 8, wherein the oxidized cellulose and structural protein (where present) together make up at least 90% by weight of the material.
- 35

10. Use according to claim 7, 8 or 9, wherein the material is a freeze-dried or solvent-dried sponge.
11. Use according to claim 7, 8 or 9, wherein the material is a solid film.
12. Use according to any of claims 7 to 11, wherein the weight ratio of protein to oxidized cellulose is from 1:99 to 99.99:1.
13. Use according to claim 12, wherein the weight ratio of protein to oxidized cellulose is in the range 1:10 to 99.9:1.
14. Use according to any of claims 7 to 13, wherein the protein comprises collagen, fibronectin, fibrin, laminin or elastin.
15. Use according to claim 14, wherein the protein consists essentially of collagen.
16. Use according to claim 14 or 15, wherein the protein comprises partially hydrolyzed, soluble collagen having molecular weights in the range 5,000-100,000.
17. Use according to claim 14 or 15, wherein the collagen is fibrous, substantially insoluble collagen.
18. Use according to any preceding claim, wherein the oxidized cellulose comprises water-soluble oxidized cellulose fragments having molecular weights in the range 5,000-50,000.
19. Use according to any preceding claim wherein said chronic wound is selected from the group consisting of venous ulcers, decubitis ulcers and diabetic ulcers.

20. A method of separating cell growth factors from a biological sample or organism, said method comprising:

- (i) contacting said biological sample or organism with a material comprising oxidized cellulose or a complex of oxidized cellulose with a structural protein, the contacting being carried out *in vivo* or *in vitro*, to bind the growth factors to the material; and
- (ii) recovering the bound growth factors from the material.

10

21. A method of preparing an active wound dressing material comprising the steps of:

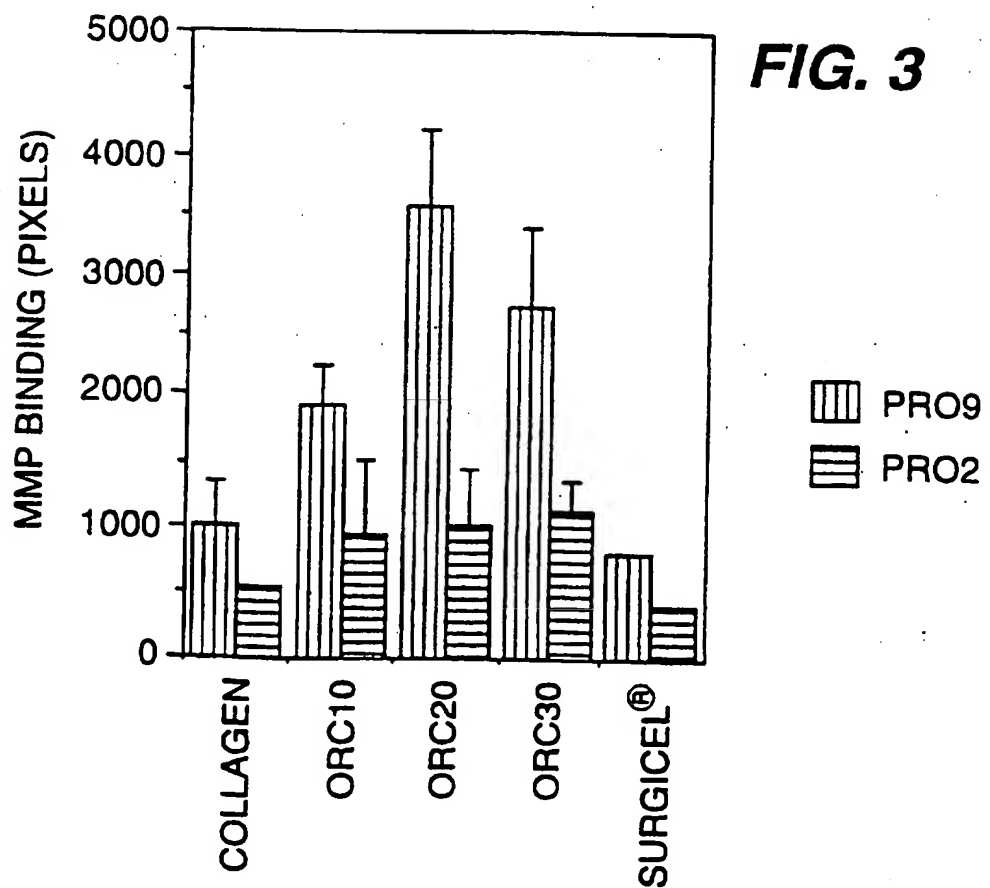
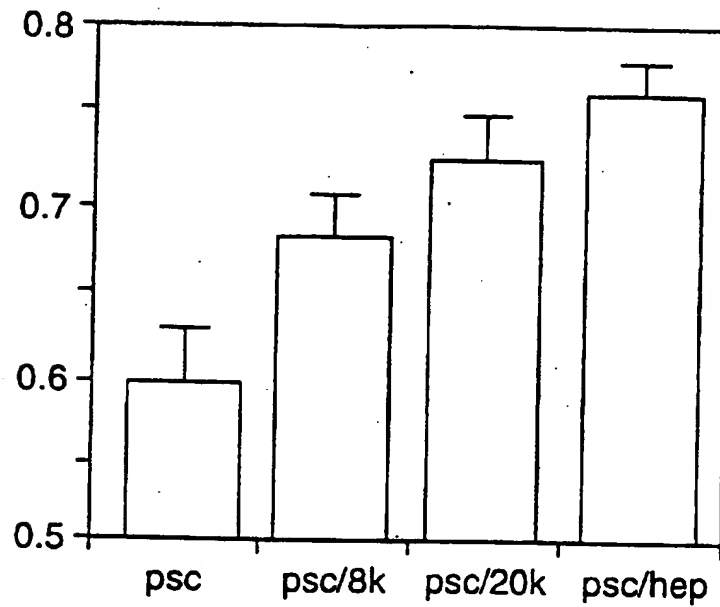
- (i) contacting a material comprising oxidized cellulose or a complex of oxidized cellulose with a structural protein with a biological medium containing cell growth factors to bind the cell growth factors to the material; and
- (ii) washing and drying the material having the cell growth factors bound thereto to form said active wound dressing material.

20

22. A method according to claim 20 or 21, wherein the cell growth factors comprise platelet derived growth factor.

23. A sterile packaged semisolid or gel ointment for topical application to a chronic wound, wherein said ointment comprises from 0.05% to 50% w/v of oxidized cellulose.

24. A bioabsorbable sponge for application to a chronic wound, said sponge comprising from 0.1% to 50% w/w of oxidized cellulose and from 50% to 99.9% of one or more structural proteins.

FIG. 1

2/3

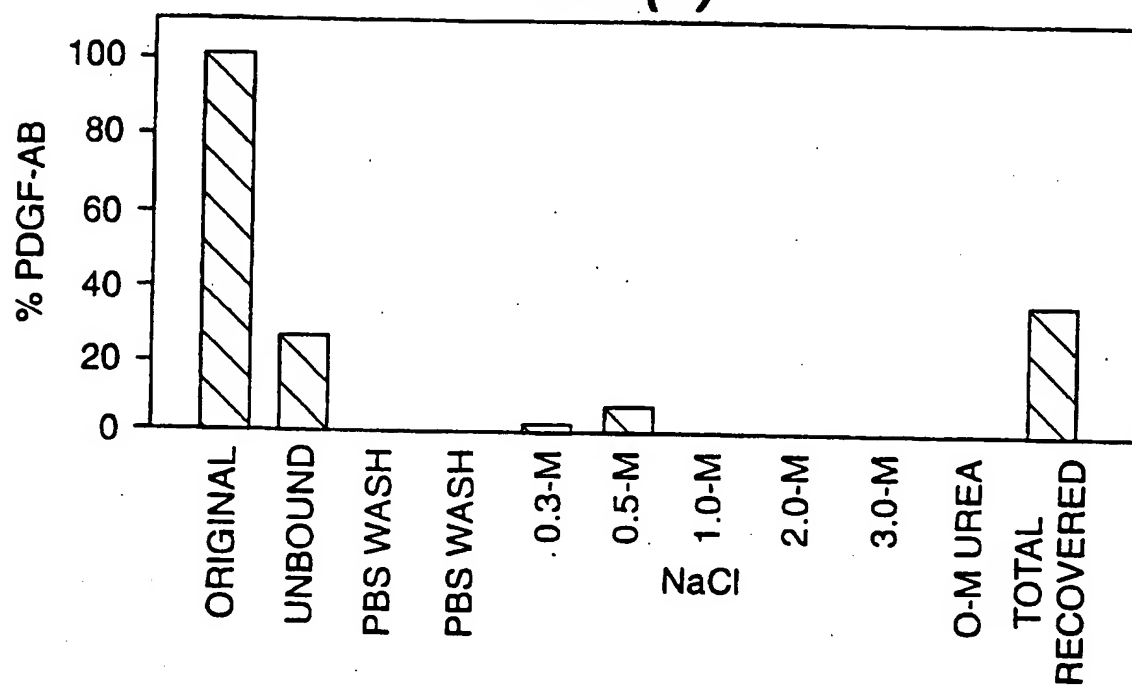
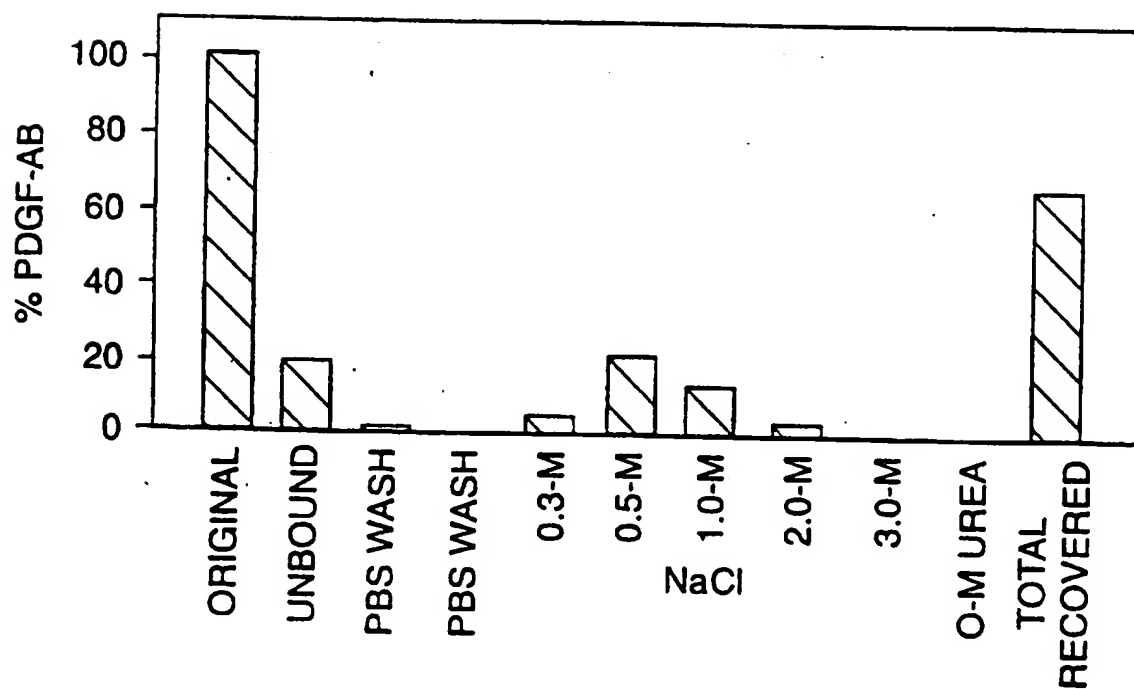
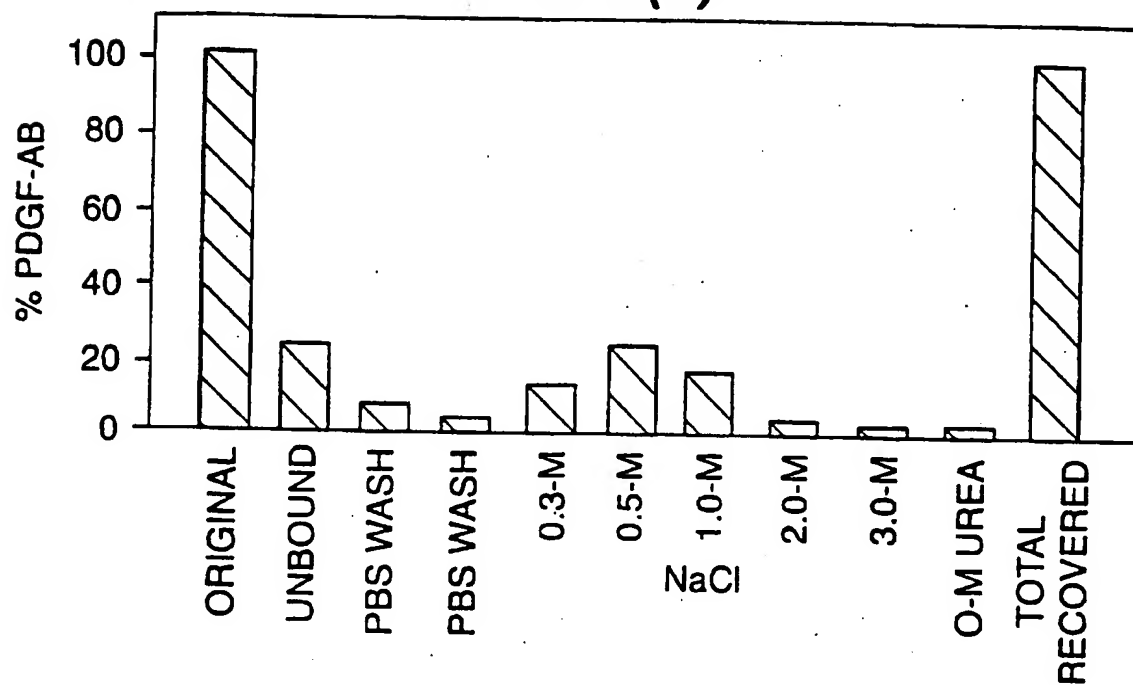
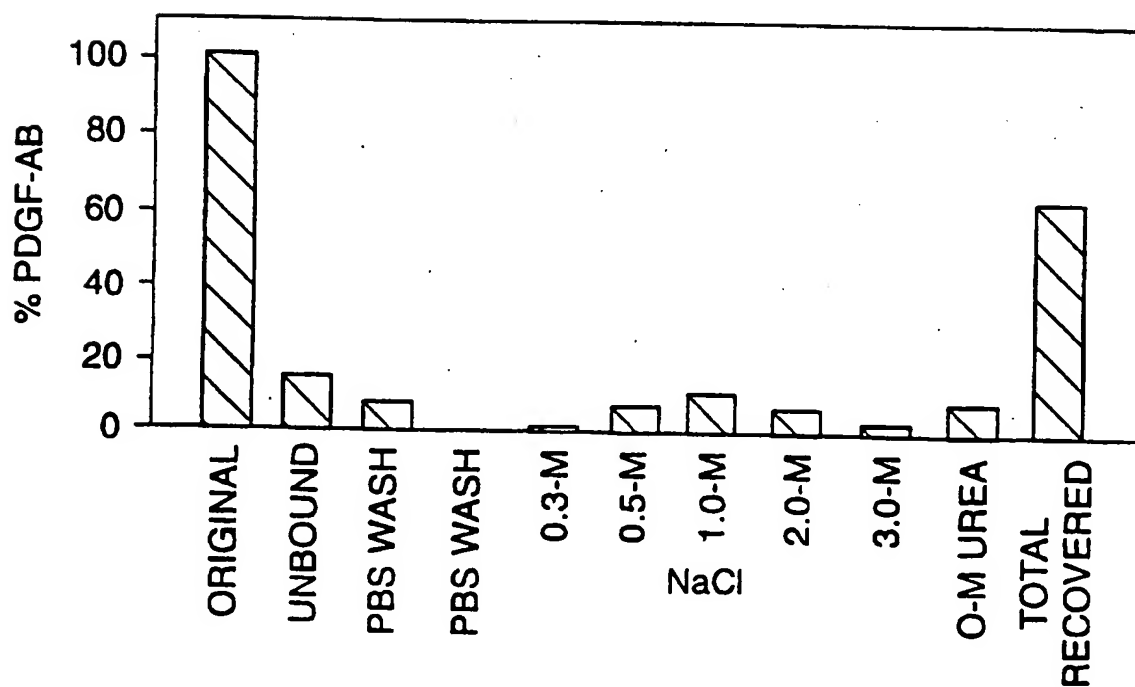
FIG. 2(a)**FIG. 2(b)**

FIG. 2(c)**FIG. 2(d)**

INTERNATIONAL SEARCH REPORT

International Application No.

GB 97/01725

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61L15/28 A61L15/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 177 064 A (JOHNSON & JOHNSON PROD INC) 9 April 1986 see claims	1,2
X	EP 0 562 862 A (JOHNSON & JOHNSON MEDICAL) 29 September 1993 see example 3	7,12-15
A	DATABASE WPI Section Ch, Week 8948 Derwent Publications Ltd., London, GB; Class A96, AN 89-348621 XP002045447 & CS 8 704 580 A (MARYSKA S), 13 October 1989 See title	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

3 November 1997

Date of mailing of the international search report

11. 11. 97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl
Fax: (+31-70) 340-3016

Authorized officer

Cousins-Van Steen, G

INTERNATIONAL SEARCH REPORT

International Application No.

P 97/01725

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 049 469 A (RUHLAND NACHF GMBH DR) 14 April 1982 ---	
A	EP 0 140 596 A (JOHNSON & JOHNSON) 8 May 1985 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
GB 97/01725

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0177064 A	09-04-86	US 4626253 A	02-12-86
		AU 4835685 A	10-04-86
		CA 1253321 A	02-05-89
		DE 3583786 A	19-09-91
		HK 17392 A	13-03-92
		JP 1866569 C	26-08-94
		JP 61090668 A	08-05-86

EP 0562862 A	29-09-93	BR 9301313 A	28-09-93
		CA 2092344 A	26-09-93

EP 0049469 A	14-04-82	DE 3037513 A	15-04-82
		AT 5373 T	15-12-83
		CA 1167726 A	22-05-84
		DE 3050567 A	05-05-83
		HK 53486 A	25-07-86
		JP 1035664 B	26-07-89
		JP 1552101 C	23-03-90
		JP 58041559 A	10-03-83
		US 4407787 A	04-10-83

EP 0140596 A	08-05-85	GB 2148901 A	05-06-85
		AU 573626 B	16-06-88
		AU 3380384 A	18-04-85
		CA 1234102 A	15-03-88
		DE 3472263 A	28-07-88
		US 4614794 A	30-09-86
